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(54) Title: **NOVEL MATRIX METALLOPROTEINASES**

(57) Abstract: The present invention provides genes encoding novel matrix metalloproteinases termed MMP; constructs and recombinant host cells incorporating the genes; the MMP polypeptides encoded by the genes; antibodies to the MMP polypeptides; and methods of making and using all of the foregoing.

homologs; kits employing the polynucleotides and polypeptides, and methods of making and using all of the foregoing. In addition, the present invention relates to homologs of the gene sequences and of the polypeptides and methods of making and using the same.

[00081] Genomic DNA of the invention comprises the protein-coding region for a polypeptide of the invention and is also intended to include allelic variants thereof. It is widely understood that, for many genes, genomic DNA is transcribed into RNA transcripts that undergo one or more splicing events wherein intron (*i.e.*, non-coding regions) of the transcripts are removed, or "spliced out." RNA transcripts that can be spliced by alternative mechanisms, and therefore be subject to removal of different RNA sequences but still encode an MMP polypeptide, are referred to in the art as splice variants which are embraced by the invention. Splice variants comprehended by the invention therefore are encoded by the same original genomic DNA sequences but arise from distinct mRNA transcripts. Allelic variants are modified forms of a wild-type gene sequence, the modification resulting from recombination during chromosomal segregation or exposure to conditions which give rise to genetic mutation. Allelic variants, like wild type genes, are naturally occurring sequences (as opposed to non-naturally occurring variants that arise from *in vitro* manipulation).

[00082] The invention also comprehends cDNA that is obtained through reverse transcription of an RNA polynucleotide encoding MMP (conventionally followed by second strand synthesis of a complementary strand to provide a double-stranded DNA).

[00083] Preferred DNA sequences encoding human MMP polypeptides are selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:3. A preferred DNA of the invention comprises a double stranded molecule along with the complementary molecule (the "non-coding strand" or "complement") having a sequence unambiguously deducible from the coding strand according to Watson-Crick base-pairing rules for DNA. Also preferred are other polynucleotides encoding the MMP polypeptide selected from the group consisting of SEQ ID NO:4 to SEQ ID NO:6, which differ in sequence from the polynucleotides selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:3, by virtue of the well-known degeneracy of the universal nuclear genetic code.

[00084] The invention further embraces other species, preferably mammalian, homologs of the human MMP DNA. Species homologs, sometimes referred to as "orthologs," in general, share at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% homology with human DNA of the invention. Generally, percent sequence "homology" with respect to polynucleotides of the invention may be calculated as the percentage of nucleotide bases in the candidate sequence that are identical to nucleotides in the MMP sequences set forth

NO: 5, or fragments thereof. In a more preferred embodiment, the nucleotide is not SEQ ID NO:7 and does not encode a polypeptide with a sequence of SEQ ID NO:8.

[00089] As used in the present invention, fragments of MMP-encoding polynucleotides comprise at least 10, and preferably at least 12, 14, 16, 18, 20, 25, 50, or 75 consecutive nucleotides of a polynucleotide encoding MMP. Preferably, fragment polynucleotides of the invention comprise sequences unique to the MMP-encoding polynucleotide sequence, and therefore hybridize under highly stringent or moderately stringent conditions only (*i.e.*, "specifically") to polynucleotides encoding MMP (or fragments thereof). Polynucleotide fragments of genomic sequences of the invention comprise not only sequences unique to the coding region, but also include fragments of the full-length sequence derived from introns, regulatory regions, and/or other non-translated sequences. Sequences unique to polynucleotides of the invention are recognizable through sequence comparison to other known polynucleotides, and can be identified through use of alignment programs routinely utilized in the art, *e.g.*, those made available in public sequence databases. Such sequences also are recognizable from Southern hybridization analyses to determine the number of fragments of genomic DNA to which a polynucleotide will hybridize. Polynucleotides of the invention can be labeled in a manner that permits their detection, including radioactive, fluorescent, and enzymatic labeling.

[00090] Fragment polynucleotides are particularly useful as probes for detection of full-length or fragments of MMP polynucleotides. One or more polynucleotides can be included in kits that are used to detect the presence of a polynucleotide encoding MMP, or used to detect variations in a polynucleotide sequence encoding MMP.

[00091] The invention also embraces DNAs encoding MMP polypeptides that hybridize under moderately stringent or high stringency conditions to the non-coding strand, or complement, of the polynucleotides set forth in sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:3.

[00092] Exemplary highly stringent hybridization conditions are as follows: hybridization at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% Dextran sulfate, and washing twice for 30 minutes at 60°C in a wash solution comprising 0.1X SSC and 1% SDS. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel *et al.* (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, *et al.*,

encodes a polypeptide comprising sequences selected from the group consisting of SEQ ID NO:4 to SEQ ID NO:6, and fragments thereof.

[000135] One preferred embodiment of the present invention provides an isolated nucleic acid molecule comprising a sequence homologous sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:3, and fragments thereof. Another preferred embodiment provides an isolated nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:3, and fragments thereof.

[000136] In a preferred embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence which encodes a polypeptide comprising a sequence of SEQ ID NO:5, or a fragment thereof. In a more preferred embodiment, the polypeptide encoded by the nucleotide sequence does not have the sequence of SEQ ID NO:8.

[000137] As used in the present invention, polypeptide fragments comprise at least 5, 10, 15, 20, 25, 30, 35, or 40 consecutive amino acids of sequences selected from the group consisting of SEQ ID NO:4 to SEQ ID NO:6. Preferred polypeptide fragments display antigenic properties unique to, or specific for, human MMP and its allelic and species homologs. Fragments of the invention having the desired biological and immunological properties can be prepared by any of the methods well known and routinely practiced in the art.

[000138] In still another aspect, the invention provides substitution variants of MMP polypeptides. Substitution variants include those polypeptides wherein one or more amino acid residues of a MMP polypeptide are removed and replaced with alternative residues. In one aspect, the substitutions are conservative in nature; however, the invention embraces substitutions that are also non-conservative. Conservative substitutions for this purpose may be defined as set out in Tables 2, 3, or 4 below.

[000139] Variant polypeptides include those wherein conservative substitutions have been introduced by modification of polynucleotides encoding polypeptides of the invention. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in Table 2 (from WO 97/09433, page 10, published March 13, 1997 (PCT/GB96/02197, filed 9/6/96), immediately below.

Table 2

Conservative Substitutions I

<u>SIDE CHAIN CHARACTERISTIC</u>	<u>AMINO ACID</u>
Aliphatic	G A P
Non-polar	I L V

incorporated herein by reference.] The analysis may entail sequencing of the entire MMP gene genomic DNA sequence, or portions thereof; or sequencing of the entire MMP coding sequence or portions thereof. In some circumstances, the analysis may involve a determination of whether an individual possesses a particular allelic variant, in which case sequencing of only a small portion of nucleic acid -- enough to determine the sequence of a particular codon characterizing the allelic variant -- is sufficient. This approach is appropriate, for example, when assaying to determine whether one family member inherited the same allelic variant that has been previously characterized for another family member, or, more generally, whether a person's genome contains an allelic variant that has been previously characterized and correlated with a mental disorder having a heritable component.

[000223] In another highly preferred embodiment, the assaying step comprises performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences. In a preferred embodiment, the hybridization involves a determination of whether nucleic acid derived from the human subject will hybridize with one or more oligonucleotides, wherein the oligonucleotides have nucleotide sequences that correspond identically to a portion of the MMP gene sequence taught herein, or that correspond identically except for one mismatch. The hybridization conditions are selected to differentiate between perfect sequence complementarity and imperfect matches differing by one or more bases. Such hybridization experiments thereby can provide single nucleotide polymorphism sequence information about the nucleic acid from the human subject, by virtue of knowing the sequences of the oligonucleotides used in the experiments.

[000224] Several of the techniques outlined above involve an analysis wherein one performs a polynucleotide migration assay, *e.g.*, on a polyacrylamide electrophoresis gel (or in a capillary electrophoresis system), under denaturing or non-denaturing conditions. Nucleic acid derived from the human subject is subjected to gel electrophoresis, usually adjacent to (or co-loaded with) one or more reference nucleic acids, such as reference MMP encoding sequences having a coding sequence identical to all or a portion of SEQ ID NOS: 1 to 3 (or identical except for one known polymorphism). The nucleic acid from the human subject and the reference sequence(s) are subjected to similar chemical or enzymatic treatments and then electrophoresed under conditions whereby the polynucleotides will show a differential migration pattern, unless they contain identical sequences. [See generally Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, New York: John Wiley & Sons, Inc. (1987-1999); and Sambrook *et al.* (eds.), *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press (1989), both incorporated herein by reference in their entirety.]

<213> Homo sapiens.

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